60. (Amended) The method of claim 59, wherein the extension of said oligonucleotide primer along said control polynucleotide is controlled by digesting the 3'-mismatch with the 3' to 5' exonuclease and extending the oligonucleotide primer along the control polynucleotide under the polynucleotide amplification conditions.

Please cancel Claim 62.

REMARKS

The foregoing amendment and the following remarks are submitted in response to the Office Action of May 22, 2002. In this Amendment, Applicant has amended Claims 1, 21, 25, 39, 59, and 60, and cancelled Claim 62. Claims 1-52, 59-61, and 63-65 are pending in the Application. Support for the amendments can be found generally throughout the Application including the drawings and claims as originally filed. Attached hereto is a marked-up version of the changes made to Claims 1, 21, 25, 39, 59, and 60 captioned "**Version With Markings To Show Changes Made**."

Objections to the Claims

Claim 21 was objected to for containing a hard return at the end of line 2. The claim has been amended and the hard return has been deleted.

Claim 62 was objected to under 37 CFR 1.75(c) for failing to further limit the subject matter of a previous claim. Claim 62 has been cancelled.

Claim Rejections Under 35 USC § 112, First Paragraph

In paragraphs 6 and 7 of the Office Action, the Examiner rejected Claims 1-57 and 59-65 under 35 USC § 112, first paragraph. Applicant will address each of the Examiner's § 112, first paragraph, rejections in turn.

In paragraph 6 of the Office Action, the Examiner states that the "claims have been limited in part to where a primer . . . encompasses modified nucleotides that are resistant to exonuclease activity, such only goes to preserve the presence of a non-extendable primer that cannot be used in the amplification of a target sequence." As mentioned throughout the Specification and recited in the claims, elongation of the primer along the control polynucleotide cannot take place until the 3' mismatch is degraded by 3' to 5' exonuclease. The modified primers disclosed and claimed are resistant to 3' to 5' exonuclease degradation, which is a prerequisite for elongation along the control polynucleotide. However, because

this degradation is not necessary for elongation along the target polynucleotide, the modified primers are extendable along the target sequence. The modified primer is not "non-extendable" and it can be used in the amplification of a target sequence.

In paragraph 7, the Office Action states that the Specification is "essentially silent as to how one is to amplify RNA[.]" Applicant respectfully directs the Examiner's attention to page 23, lines 11-14, which defines polynucleotide analytes as including "nucleic acids from any source in purified or unpurified form including . . . RNA, including t-RNA, m-RNA, r-RNA, mitochondrial . . . RNA, chloroplast . . . RNA, [and] DNA-RNA hybrids[.]" Applicant further directs the Examiner's attention to page 61, lines 1-2, of the Specification which states "[t]he present method has application where the target polynucleotide sequence is . . . RNA." Finally, Applicant respectfully directs the Examiner's attention to page 62, lines 9-10, of the Specification, which states "[w]here the polynucleotide analyte is RNA, the nucleotide polymerase comprises a reverse transcriptase."

In paragraph 7, the Office Action further states that the Specification is "essentially silent as to how one is to . . . generate RNA amplicons[.]" Applicant respectfully directs the Examiner's attention to page 32, lines 15-21 of the Specification, which mentions two methods for generating RNA amplicons: nucleic acid sequence based amplification ("NASBA") and the Q-beta-replicase method.

Claim Rejections Under 35 USC § 112, Second Paragraph

Claims 1-52 and 59-65 were rejected under 35 USC § 112, second paragraph.

In paragraph 10 of the Office Action, the Examiner rejected Claim 1 and Claims 2-8 under 35 USC § 112, second paragraph. The claim has been amended to define the relation of the "target sequence" to the "oligonucleotide primer" more precisely, and Applicant requests that the rejection for Claim 1 be withdrawn. The Examiner rejected Claims 2-8 as being dependent on rejected Claim 1. Applicant respectfully points out that Claim 2 is an independent claim, and that Claims 3-8 depend from Claim 2, and requests that the rejections for Claims 2-8 be withdrawn.

In paragraph 11 of the Office Action, the Examiner rejected Claims 3, 9, 26, and 40 under 35 USC § 112, second paragraph, for use of the term "fully complementary" and failure to distinguish the term from the term "complementary." (Applicant respectfully points out that the term "fully complementary" does not appear in rejected Claim 9, but rather in its dependent Claim 10.) Applicant respectfully submits that the plain language of Claims 3, 9, 10, 26, and 40 is definite, and that the term "fully complementary" is used in Claims 3, 10, 26, and 40 to emphasize the contrast to "complementary . . . except for said 1 to 10 nucleotides at the 3'-end thereof." Applicant respectfully submits that every

pending claim in the instant Application includes the limitation that the oligonucleotide primer contains a 3'-mismatch to a control (or "second") polynucleotide, and that Claims 3, 10, 26, and 40 merely provide a further limitation on the quality of the match between the primer and the sequence to which it hybridizes. The claims as written are definite and the rejection should be withdrawn.

In paragraph 12 of the Office Action, the Examiner rejected Claim 9 under 35 USC § 112, second paragraph. In his rejection the Examiner states that the claim is "indefinite with respect to how a first primer can, in essence, hybridize to self." Specifically, the Examiner states that the indefinite language in Claim 9 is with respect to how a "first primer" can be "capable of hybridizing to" an "extended first primer." This language is designed to encompass the case where the target sequence is flanked by identical priming sequences. In this situation, only one primer is necessary for amplification. (One of ordinary skill in the art recognizes that the phrase "one primer" means a plurality of identical primers. Indeed, as disclosed in the example in the Specification on page 75, line 15, to page 76, line 11, the elongation reaction occurs in a total volume of 100 microliters, with a primer concentration of 1.0 micromolar. The reaction therefore utilizes approximately $6.02 * 10^{14}$ copies of the same primer.) In the situation where the target sequence is flanked by identical primer sites, the first primer will indeed be able to hybridize to and extend along an extended first primer. The language is definite to one of ordinary skill in the art and the rejection should be withdrawn.

In paragraph 13, the Examiner states that dependent Claim 23 is confusing because Claim 23 recites the limitation that the first and second primers are different, implying that they do not have to be different in parent Claim 9. Claim 24 recites a similar limitation. As explained in the above paragraph, if the target sequence has identical flanking sequences, then the first and second primers can indeed be identical. Similarly the rejection of Claims 23 and 24 under 35 USC § 112, second paragraph, in paragraph 15 of the Office Action should be withdrawn.

In paragraph 14 of the Office Action, the Examiner rejected Claims 4 and 41 under 35 USC § 112, second paragraph. The Examiner also rejected Claims 5 and 6, which depend from Claim 4, and "claim 41, which depends from claim 41 [sic]." Applicant will presume that the Examiner meant to reject Claims 42 and 43, which depend from Claim 41. In his rejection, the Examiner stated that the phrase "substantially identical" was indefinite. Applicant respectfully directs the Examiner's attention to Applicant's Amendment After Final, submitted on February 27, 2002, in which Claims 4, 9, 27, and 41 were amended to delete the word "substantially." The claims as amended in the February 27, 2002 Amendment After Final are in condition for allowance.

In paragraph 16 of the Office Action, the Examiner rejected Claim 25 for insufficient definition of "control primer." Claim 25 has been amended to indicate that the control primer is selected from a Markush group consisting of primer A and primer B, and is in condition for allowance.

In paragraph 17 of the Office Action, the Examiner rejected Claim 39 for indefinite use of the phrase "partially sequentially". The claim has been amended and the phrase has been removed.

In paragraph 18 of the Office Action, the Examiner rejected Claims 59 and 60 for indefinite use of the term "further controlled." The claims have been amended to address this rejection by eliminating the word "further," and are in condition for allowance.

In paragraph 19 of the Office Action, the Examiner rejected Claim 63, stating that the claim appears to be an incomplete sentence. Applicant respectfully submits that the claim as written is a complete sentence, providing two further limitations to the recited "target sequence" element. The first limitation is that the target sequence is single stranded. The second limitation is that the target sequence contains inverted repeats which hybridize to the primer. The rejection should be withdrawn.

Claim Rejections Under 35 USC § 103

Claims 1-3, 7-10, 14-26, 30-40, 44-52, 59, 60, and 62-65 were rejected under 35 USC § 103(a) as being unpatentable over Sorenson (WO 93/22456), Gelfand et al. (U.S. Pat. No. 5,491,086), and Barnes (U.S. Pat. No. 5,436,149). The Examiner states that Sorenson discloses the use of a plurality of primers that vary at their 3' end, that Gelfand discloses the use of a DNA polymerase with a 3' to 5' exonuclease activity, and that Barnes discloses the use of exonuclease in amplification reactions to remove primer mismatches from the 3' end.

To establish a prima facie case of obviousness based on a combination of the content of various references, there must be some teaching, suggestion or motivation in the prior art to make the specific combination that was made by the applicant. *In re Dance*, 160 F.3d 1339, 1342 (Fed. Cir. 1998); In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1445 (Fed.Cir.1992). When the motivation to combine the teachings of the references is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper. *Ex parte Skinner*, 2 USPQ2d 1788 (Bd. Pat. App. & Inter. 1986). Neither the cited references, nor the prior art, contain a teaching, suggestion or motivation to make the specific combination made by the Applicant.

Sorenson discloses the use of a plurality of primers for the detection of mutant alleles. Sorenson, pages 3-5. Specifically, Sorenson discloses the use of allele-specific primers to detect the presence of known mutant alleles. For example, Sorenson's Claim 1 recites the use of "one primer complementary

to a mutation-containing segment." Sorenson does not contain any teaching, suggestion, or motivation to make the specific combination that was made by Applicant. In fact, by disclosing primers designed for specific matches, Sorenson teaches away from the present invention which discloses and claims primers designed for their mismatches. Sorenson does not disclose or claim the extension or amplification of mismatched primers, but rather teaches away from it. In paragraph 23 of the Office Action, the Examiner draws Applicant's attention to page 11 of Sorenson, which states that "only the primer which is fully complementary to the allele which is present will anneal and extend." Here again, Sorenson teaches away from the present invention, where the mismatched primer will anneal to the control polynucleotide and extend once the 3'-mismatch has been degraded. In paragraph 24, the Examiner admits that Sorenson does not disclose using exonuclease activity. In fact, Sorenson teaches away from the present invention on this point. On page 5, second paragraph, Sorenson states: "The PCR techniques described above preferably use a DNA polymerase which lacks a 3' exonuclease activity." By contrast, in several embodiments of the instant Invention, 3' exonuclease activity is required for the control polynucleotide to be amplified.

Gelfand discloses thermostable DNA polymerases and means for isolating and producing the enzymes. Gelfand discloses that the native, wild-type, thermostable DNA polymerase isolated from Pyrodictum contains 3'-5' exonuclease activity. Gelfand, column 15, line 29-31. Gelfand notes that 3'-5' exonuclease activity may be "generally" desirable for its proofreading effects. Lines 32-37, and Example 6, columns 26-27. Gelfand does not contain any teaching, suggestion, or motivation to make the specific combination that was made by Applicant. In fact, Gelfand here teaches away from the present Invention, because he discloses the use of 3'-5' exonuclease for the removal of misincorporated bases of the synthesized polynucleotide, but does not disclose the use of an exonuclease to remove deliberate mismatches designed in the primer. Gelfand further notes that in the context of PCR amplification, 3'-5' exonuclease activity is considered undesirable for several reasons, including increased non-specific background amplification, as well as non-specific degradation of primers and target polynucleotides. Column 15, lines 37-48. In Example 7, columns 28-29, Gelfand discloses the production of a recombinant thermostable DNA polymerases which lack 3'-5' exonuclease activity. Applicant respectfully submits that by disclosing a wild-type polymerase with 3'-5' exonuclease activity, and the generation of recombinant polymerases for the specific purpose of removing exonuclease activity, Gelfand teaches away from the present Invention, which utilizes 3'-5' exonuclease activity in several of its embodiments.

Barnes discloses the addition of a small amount of thermostable Pfu DNA polymerase to increase the efficiency of a PCR reaction. Barnes, column 17, lines 24-46. Pfu polymerase has 3'-5' exonuclease activity. In an especially preferred embodiment, Barnes discloses the use of a ratio of 640 units of KlenTaq-278 (a 3'-exonuclease-free polymerase) to 1 unit Pfu. Barnes does not contain any teaching, suggestion, or motivation to make the specific combination that was made by Applicant. Similar to Gelfand, Barnes discloses that a benefit of 3'-5' exonuclease activity is the removal of misincorporated bases during elongation. Barnes speculates that the increased efficiency is due to the removal of misincorporated bases by the Pfu because mismatches cause pauses in elongation. Barnes does not disclose, nor contain any teaching, suggestion, or motivation to use a 3'-5' exonuclease to degrade a primer.

The above-cited references do not contain any teaching, suggestion, or motivation to make the specific combination that was made by Applicant, and teach away from the present Invention in several important respects noted above. The Examiner, by merely stating in paragraph 29 of the Office Action that an "ordinary artisan would have been highly motivated" to combine the references, has not met his burden under *Skinner* and explained why the combination of the references is proper. Nor has the Examiner addressed the significant ways noted above that the references teach away from the specific combination of the present Invention. Applicant respectfully requests the rejection be withdrawn.

CONCLUSION

Applicant respectfully requests entry of the foregoing amendments. Applicant believes that all pending claims are in condition for allowance, and respectfully requests that all rejections be withdrawn and that all pending claims be allowed.

Respectfully submitted,

Michael Schmelzer, Reg. No. 43,093
Attorney/Agent for Applicant

Aventis Pharmaceuticals Inc.
Patent Department
Route #202-206 / P.O. Box 6800
Bridgewater, NJ 08807-0800
Telephone (908) 231-4797
Telefax (908) 231-2626

Version With Markings To Show Changes Made

1. (Twice Amended) In a method for forming multiple copies of a target sequence of a target polynucleotide, said method comprising the steps of combining a sample with reagents for forming said multiple copies, subjecting said sample to polynucleotide amplification conditions sufficient to form the multiple copies if the target sequence is present in said sample, said reagent comprising an oligonucleotide primer and polymerase; and forming an extension product[s] of the oligonucleotide primer at least along said target sequence [or along an extended nucleotide primer complementary to said oligonucleotide primer], said extension product[s] being [copies] a complement of said target sequence, the improvement which comprises forming said extension product in the presence of a [second] control polynucleotide to which said oligonucleotide primer hybridizes except for a 3'-mismatch on said oligonucleotide primer, under polynucleotide amplification conditions wherein the extension of said oligonucleotide primer along said [second] control polynucleotide is controlled by the 3'-mismatch relative to the extension of said oligonucleotide primer along said target sequence.

21. (Amended) The method of Claim 9 wherein said extending is carried out in the [ ] presence of nucleoside triphosphates.

25. (Twice Amended) A method for forming multiple copies of at least one double stranded polynucleotide ("polynucleotide") said polynucleotide comprising a single stranded target polynucleotide sequence ("target sequence") and its complementary sequence ("complementary sequence"), said method comprising:

(a) treating a sample suspected of containing one or more of said double stranded polynucleotides with (i) at least two oligonucleotide primers capable of hybridizing to a portion of each target sequence and its complementary sequence suspected of being present in said sample under polynucleotide amplification conditions for hybridizing said primers to and extending said primers along said target sequence and said complementary sequence[s], wherein said primers are selected such that the extension product formed from one primer ("primer A"), when it is dissociated from its complement, can serve as the template for the extension product of another primer ("primer B"), (ii) a control polynucleotide, as a template to which [either primer A or B] a control primer hybridizes except for 1-10 nucleotides of the primer at the 3'-end [(control primer)], wherein said control primer is selected from the group consisting of primer A and primer B, and (iii) a 3' to 5' exonuclease wherein said primers extend, respectively, along said

target sequence and said complementary sequence and the control primer extends along said control polynucleotide only after said 1-10 nucleotides are degraded by said 3' to 5' exonuclease,

(b) dissociating primer extension products from their respective templates to produce single stranded molecules and

(c) treating the single stranded molecules produced in step (b) with the primers of step (a) under polynucleotide amplification conditions such that a primer extension product is formed using the single strands produced in step (b) as templates, resulting in amplification of the target sequences and complementary sequences if present, said polynucleotide amplification conditions allowing for the extension of the control primer along said control polynucleotide to provide said positive internal control.

39. (Twice Amended) A method of producing multiple copies of a target sequence of a target polynucleotide, which comprises:

(a) providing in combination (1) a single stranded polynucleotide having a sequence that is said target sequence and that is flanked at each end by at least partially complementary first and second flanking sequences, (2) an oligonucleotide primer at least a 10 base portion of which at its 3'-end is hybridizable to that member of said first and second flanking sequences that is at the 3'-end of said single stranded polynucleotide, (3) nucleoside triphosphates, (4) a control polynucleotide, as a template to which said oligonucleotide primer hybridizes except for 1-10 nucleotides at the 3'-end of said nucleotide primer, and (5) a 3' to 5' exonuclease wherein said primer extends along said target sequence and said primer extends along said control polynucleotide only after said 1-10 nucleotides are degraded by said 3' to 5' exonuclease,

(b) incubating said combination under polynucleotide amplification conditions for [either wholly or partially sequentially or concomitantly] (1) dissociating said single stranded polynucleotide from any complementary sequences, (2) hybridizing said oligonucleotide primer with the flanking sequence at the 3'-end of said single stranded polynucleotide and with said control polynucleotide, (3) extending said oligonucleotide primer along said single stranded polynucleotide to provide a first extended oligonucleotide primer and degrading said oligonucleotide primer hybridized to said control polynucleotide and extending said degraded oligonucleotide along said control polynucleotide, (4) dissociating said first extended primer and said single stranded polynucleotide and dissociating said control polynucleotide and said extended degraded primer, (5) hybridizing said first extended oligonucleotide primer with said oligonucleotide primer and hybridizing said oligonucleotide primer and said control

polynucleotide, (6) extending said oligonucleotide primer along said first extended oligonucleotide primer to provide a second extended oligonucleotide primer and degrading said oligonucleotide primer hybridized to said control polynucleotide and extending said oligonucleotide primer along said control polynucleotide to provide an extended degraded primer, (7) dissociating said second extended oligonucleotide primer from said first extended oligonucleotide primer and said extended degraded primer from said control polynucleotide, and (8) repeating steps (5)-(7) above, and

(c) detecting the presence of said extended degraded primer, the presence thereof indicating that said reagents and polynucleotide amplification conditions for producing multiple copies of said target sequence of a target polynucleotide are functional.

59. (Amended) The method of claim 1, wherein the extension of said oligonucleotide primer along said control polynucleotide is [further] controlled [along said second polynucleotide] by contacting the 3'-mismatch with a 3' to 5' exonuclease.

60. (Amended) The method of claim 59, wherein the extension of said oligonucleotide primer along said control polynucleotide is [further] controlled by digesting the 3'-mismatch with the 3' to 5' exonuclease and extending the oligonucleotide primer along the [second] control polynucleotide under the polynucleotide amplification conditions.